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Genetics

Yeast recombination: The association between double-strand gap repair and crossing-over

(gene conversion/yeast transformation/plasmid integration/double-strand break repair/chromosomal allele recovery)

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ABSTRACT **In previous experiments, we have used yeast transformation to study the recombinogenic repair of double-strand breaks and gaps. A plasmid containing a double-strand gap within sequences homologous to the yeast genome integrates efficiently by crossing-over. During the process of integration, the double-strand gap is repaired, using chromosomal information as a template. This repair reaction results in the transfer of genetic information from one DNA duplex to another and is therefore a pathway for gene conversion. Because meiotic gene conversion is associated with a high frequency (up to 50%) of crossing-over, we wished to determine the degree of association of double-strand gap repair with crossing-over. Only the class of repair events resulting in crossovers (plasmid integration) were detected in our earlier experiments with nonreplicating plasmids. In this paper, we describe the outcome of double-strand gap repair in plasmids that are capable of autonomous replication and therefore allow recovery of both crossover and noncrossover products. After the correct repair of a double-strand gap, we recover approximately equal numbers of integrated and nonintegrated plasmids. Thus, gene conversion by double-strand gap repair can occur either with or without crossing-over, and it is similar in this respect to meiotic gene conversion. Circularization of linear plasmid DNA by ligation is also observed, suggesting that yeast has an additional repair pathway for double-strand breaks that is independent of recombination. Gap repair on replicating plasmids permits rapid cloning of chromosomal alleles.**

We have previously used yeast transformation with nonreplicating plasmids that integrate into the genome by homologous recombination as a model system for the study of recombination (1). We showed that double-strand breaks are recombinogenic; linear plasmids containing a double-strand break within yeast DNA sequences transform at much higher frequencies than uncut plasmids. Moreover, a complex plasmid containing fragments from more than one location in the yeast genome can be directed to integrate at a specific site in the genome by making a double-strand break within the corresponding fragment on the plasmid. A plasmid that has a double-strand gap also transforms efficiently, and the gap is repaired from chromosomal sequences during plasmid integration. The *RAD52* gene product is required for the integration of linear or gapped, but not circular, plasmids.

Gene conversion is the nonreciprocal transfer of information from a donor to a recipient DNA duplex. Chromosomal information is used in the repair of a double-strand gap on a plasmid and markers lying within the gap are converted (2). Double-strand gap repair is therefore potentially a mechanism for gene conversion. We have recently proposed that meiotic recombination is initiated by double-strand breaks (3). In our model, gene conversion is due to the repair of double-strand gaps produced by degradation from double-strand breaks. Gene conversion in meiosis is associated with a high frequency of crossing over, such that markers flanking the conversion site are exchanged (4). We therefore wished to determine whether gap repair in mitotic cells occurs with or without crossing-over. In transformation, an integrated plasmid is produced when the repair of a gap is accompanied by a crossover, while repair without an associated crossover yields a nonintegrated plasmid (see Fig. 1). Because nonreplicating plasmids were used in our earlier experiments, only those repair events that resulted in crossovers were detected.

Plasmids that contain a chromosomal *ARS* (autonomously replicating sequence) transform yeast at high frequencies (5) and are maintained extrachromosomally. Because *ARS* plasmids produce transformants without requiring integration into chromosomal DNA, repair of a gap on an *ARS* plasmid can be observed, whether or not it occurs with an associated crossover. In this paper, we report that, after correct repair of a double-strand gap on an *ARS* plasmid, we recover similar numbers of integrated (crossover) and nonintegrated (noncrossover) plasmids. Thus, gene conversion via double-strand gap repair on plasmids behaves analogously to meiotic gene conversion with respect to exchange of outside markers.

MATERIALS AND METHODS

Strains and Media. Strain LL20 (*α, can1, his3-11,15, leu2-3,112*) was obtained from Lester Lau. Strain D234-3B (*α, his3-11,15 leu2-3,112, tcm1, trp1, ura3*) was constructed in this laboratory by Patricia Brown. The *ura3* allele was isolated after mutagenesis with ethylmethanesulfonate (6). Strain T1128 is a transformant of D234-3B, which has a single copy of pSZ515 integrated at the *his3* locus. Media were prepared as described (6). The DNA from transformants containing unstable replicating plasmids was isolated from cells grown under selection for plasmid-borne markers.

Yeast Transformation. Yeast transformation was as described (2). Carrier DNA was not used in any of these transformations. In contrast to our previous results, which showed that linear or gapped integrating plasmids transform at higher frequencies than do circular plasmids, linear or gapped replicating plasmids transform at frequencies that are 1–20% of those of circular replicating plasmids. We routinely used 10 μg of linear or gapped plasmid DNA and 0.5 μg of circular plasmid DNA per transformation. Transformants containing extrachromosomal plasmids were distinguished from those with integrated plasmids by the mitotic stability of selective markers present on the plasmid. A transformant colony was streaked out to single colonies on nonselective medium, replica plated to

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Abbreviations: bp, base pair(s); kb, kilobase(s).

selective medium, and checked for growth. In stable transformants, >99% of the colonies grow on the selective plate, compared with <10% of the colonies from an unstable transformant.

Plasmid Constructions, DNA Purification, and Southern Blots. Plasmid construction, restriction digestion of plasmid DNA, purification of plasmid and yeast DNA, and Southern blots have been described (2). The 60-base-pair (bp) *Bgl* II fragment from *HIS3* (R. Rothstein, personal communication) was 5′-end labeled for use as a hybridization probe. pSZ64 (1) was digested with *Bgl* II, 5′-end labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (7), and fractionated on a 6% polyacrylamide gel, and the 60-bp fragment was electroeluted and ethanol precipitated.

RESULTS

Association of Double-Strand Gap Repair with Crossing-Over. Gap repair accompanied by crossing-over leads to the integration of a plasmid. Since plasmids bearing an *ARS* element replicate autonomously (5), we determined the degree of crossing-over accompanying gap repair by transforming yeast with an *ARS* plasmid with a double-strand gap, selecting for gap repair, and comparing the fraction of transformants containing integrated versus autonomous plasmids (Fig. 1). Integrated plasmids can be distinguished from extrachromosomal plasmids by the mitotic stability conferred on the transformant for selectable markers present on the plasmid.

A 60-bp double-strand gap was made within the *his3* structural gene on the *ARS1, LEU2, his3* plasmid pSZ511 by digestion with *Bgl* II (Fig. 2). pSZ511 is a deletion derivative of pSZ212 generated by digesting pSZ212 with *Bgl* II and religating. Therefore cleavage of pSZ511 at its single *Bgl* II site produces a gapped molecule. Gapped pSZ511 was transformed into the his$^-$ strain LL20 and recombinants in which the gap had been correctly repaired were obtained by selecting HIS$^+$ transformants. Since the chromosomal *his3* mutations in LL20 lie outside the region of the gap, HIS$^+$ transformants can arise by repair of the gap from the chromosomal *his3* DNA. We obtained 69 stable and 69 unstable HIS$^+$ transformants after transformation with 10 μg of DNA; all were LEU$^+$. In contrast, 68 clones transformed with circular pSZ511 all showed an unstable LEU$^+$ phenotype (Table 1). Thus, integration of pSZ511 is coupled with gap repair. We have also observed similar levels of crossing-over associated with the repair of other double-strand breaks

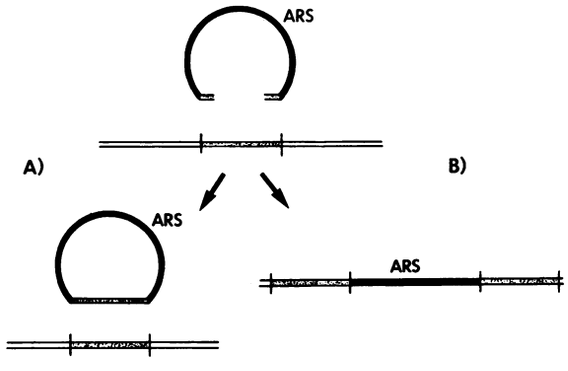

FIG. 1. Repair of a gapped replicating plasmid. The double-strand gap on a replicating plasmid containing a chromosomal *ARS* is repaired from homologous chromosomal sequences. Gap repair without crossing-over produces a repaired replicating plasmid (*A*); repair accompanied by a crossover results in an integrated plasmid (*B*). ▨, Homologous DNA on the plasmid and chromosome; ■, plasmid DNA; □, chromosomal DNA.

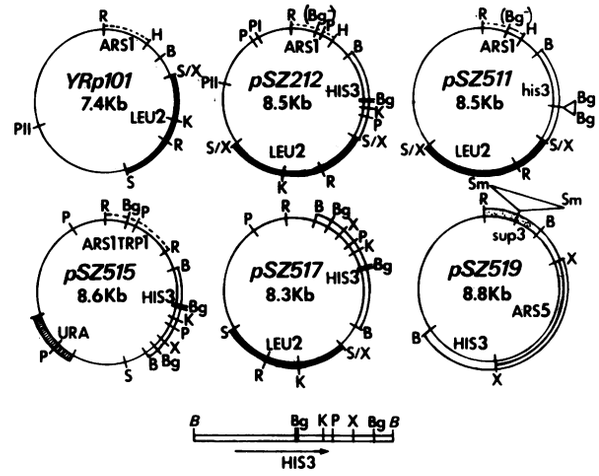

FIG. 2. Restriction maps of plasmids and the *HIS3* fragment. YRp101 contains the *ARS1* and *LEU2* genes. pSZ212 (8) contains *ARS1, LEU2,* and *HIS3*. The *Bgl* II site in *ARS1* has been removed by bisulfite mutagenesis. A *his3* deletion derivative, pSZ511, was constructed by digestion of pSZ212 with *Bgl* II and religation under dilute conditions. pSZ515 contains the 1.7-kilobase (kb) *Bam*HI fragment from pSZ62 (1) inserted into pJT29, an *ARS1* plasmid with *URA3* G-C tailed into the *Ava* I site of pBR322 (J. Thomas, Massachusetts Institute of Technology). The integrating *LEU2 HIS3* plasmid pSZ517 was constructed by transferring the 2.3-kb *LEU2* fragment from pYeLEU10 (9) into the *Sal* I site of pSZ62. pSZ519 contains a 1.2-kb deletion in the *sup3*$^+$ fragment of pWJ52. pWJ52 consists of pWJ12 (1) with a 2.6-kb *ARS5* fragment inserted into the *Xho* I site of the *HIS3* fragment. pWJ52 was digested with *Sma* I and religated, producing pSZ519. The arrow below the restriction map of the *Bam*HI *HIS3* fragment designates the position and direction of the *HIS3* transcript (10). The *his3-11,15* alleles lie between the two closely spaced *Bgl* II sites and the 5′ end of the gene (1). B, *Bam*HI; Bg, *Bgl* II; H, *Hin*dIII; K, *Kpn* I; P, *Pst* I; PI, *Pvu* I; PII, *Pvu* II; R, *Eco*RI; S, *Sal* I; Sm, *Sma* I; X, *Xho* I; ——, pBR322; ■, ▨, □, ▩, ▤, ☰, yeast DNA, as indicated.

and gaps in the *HIS3* gene and with the repair of a gap in a *sup3*$^+$ fragment (Tables 2 and 3).

Gap repair with an associated crossover and the generation of an integrated plasmid is observed only if the double-strand break or gap is in yeast DNA. The interaction between homologous chromosomal and plasmid DNA appears to be required because linear plasmids with double-strand breaks in pBR322 rarely produce stable transformants with integrated plasmid DNA (Table 1). Therefore, gap repair and recombination are products of the same event.

Transformation with Linear Replicating Plasmids Does Not Stimulate Excision of Integrated Plasmids. Although gap repair generates both repaired extrachromosomal and integrated plasmids, it is possible that all gap repair proceeds through integration and that replicating plasmids result from subsequent excision of plasmid DNA. To test whether gap repair stimulates excision of an integrated plasmid, we constructed strain T1128. This strain contains a single copy of pSZ515, which bears *ARS1 HIS3* and the selectable marker *URA3*, integrated at the *his3* gene of strain D234-3B. T1128 was transformed with replicating plasmids and with integrating plasmids that either were uncut, had breaks or gaps in pBR322, or contained breaks or gaps in yeast DNA. We selected transformants that retained pSZ515 (were URA$^+$) and received the second plasmid. If the integrated copy of pSZ515 could be excised, the transformant would become unstable for the URA$^+$ marker. For each species of transforming DNA, we tested the stability of the URA$^+$ marker in 48 transformants. In all cases, each of the 48 transformants examined was stable URA$^+$. The DNA species used were (*i*) circular or linear YRp101, an *ARS1 LEU2* plasmid (Fig. 2); (*ii*)

Table 1. Phenotypes of pSZ511 and pSZ212 transformant colonies

Plasmid	Site	$LEU^{+u}HIS^{+u}$	$LEU^{+u}his^{-}$	$LEU^{+s}HIS^{+s}$	$LEU^{+s}his^{-}$	$LEU^{+u}HIS^{+s}$	$LEU^{+s}HIS^{+u}$	% his^{-}*
Uncut pSZ511	—	0	68	0	0	0	0	100
Bgl II-cut pSZ511	*HIS3*	69	—	66	—	3	0	—
Uncut pSZ212	—	32	0	0	0	0	0	0
Pvu II-cut pSZ212	pBR322	21	0	0	0	0	0	0
*Bam*HI-cut pSZ212	pBR322	124	0	1	2	0	0	2
Bgl II-cut pSZ212	*HIS3*	15	88	4	21	0	2	84

Since small and large transformant colonies appear to pick colonies randomly and without bias, all transformants from one region of each transformation plate were checked. In general, the small colonies are unstable transformants and the large are stable. Selection was for LEU^{+} except for *Bgl* II-cut pSZ511, in which case HIS^{+} transformants were selected. u, Unstable transformant; s, stable transformant.
* Percentage of his^{-} transformants.

circular or linear pSZ32 (1), which integrates at *rDNA*; (*iii*) pSZ511 uncut or cut in the *HIS3* fragment; and (*iv*) pSZ517 (Fig. 2) uncut or targeted to integrate at *HIS3*. Since none of these transforming DNAs stimulate the excision of an integrated plasmid, repaired autonomous plasmids are unlikely to be due to excision caused by secondary recombination events.

Additional Classes of Gap Repair Events. After repair of gapped pSZ511, we obtained equal numbers of transformants having integrated and nonintegrated plasmids. By selecting HIS^{+} transformants in this experiment, we demanded that the gap be correctly repaired from chromosomal information. Analysis of transformants in which correct gap repair was not selected revealed three additional classes of gap repair events: (*i*) transfer of chromosomal alleles flanking the break or gap to the plasmid by gene conversion, (*ii*) partial gap repair, and (*iii*) religation of homologous overhanging ends.

To characterize gap repair in the absence of selection for correct repair, we transformed yeast with pSZ515, which contains *ARS1*, *TRP1*, *HIS3*, and *URA3*. A double-strand break or gap was made in the *HIS3* gene, but URA^{+} transformants were selected. The percentage of stable integrated transformants ranged from 20% to 40%, and we observed that transformants were frequently his^{-} (Table 2). his^{-} transformants that are stable for uracil prototrophy could presumably arise by a substitution event at the *URA3* locus (11). In such substitution events, the chromosomal allele of the selected gene is replaced by the corresponding plasmid information without gap repair and plasmid integration. However, the majority of his^{-} transformants did contain the plasmid, as shown by Southern blotting. Furthermore, two types of his^{-} transformants were obtained, those that were stable and those unstable for URA^{+}. Therefore, transformants containing a his^{-} plasmid could arise either by imprecise gap repair or by gene conversion of the chromosomal *his3* mutations to the plasmid. The *his3-11,15* mutations lie between the most 5′ *Bgl* II site and the 5′ end of the gene (Fig. 2); a gene conversion event in this region would result in a his^{-} plasmid. One way in which conversion could occur is by degradation of the linear plasmid DNA through the region homologous to the chromosomal mutations. The plasmid gap would then cover the mutations and gap repair would transfer the mutations to the plasmid (2). Alternatively, gene conversion could occur by the formation and subsequent repair of heteroduplex DNA. In our model for gap repair, each 3′ end flanking the gap invades homologous chromosomal DNA, producing two regions of heteroduplex DNA adjacent to the gap (1).

We analyzed the structure of the pSZ515 DNA in transformants obtained from the *Kpn* I/*Xho* I-gapped plasmid by Southern blot restriction mapping to determine whether the gap was accurately repaired. The criterion for correct gap repair was the regeneration of the *Pst* I restriction enzyme site inside the gap (Fig. 2). Ten stable URA^{+} HIS^{+} and 10 stable URA^{+} his^{-} transformants were analyzed by Southern blots and each contained all four *Pst* I sites present in pSZ515 (Fig. 3 *Upper*). Sixteen unstable URA^{+} HIS^{+} and three unstable URA^{+} his^{-} transformants also were repaired to regenerate the *Pst* I site inside the *Kpn* I/*Xho* I gap (Fig. 3 *Lower*). These transformants contain a full length 1.7-kb *Bam*HI *HIS3* fragment (data not shown). Thus, correct gap repair had occurred in 13 his^{-} transformants analyzed; the chromosomal mutations have been transferred to the plasmid. The plasmids do not appear to have become his^{-} as a result of gap repair being error prone; we have been unable to obtain HIS^{+} mitotic recombinants after irradiation of four of the stable URA^{+} his^{-} transformants. Transfer of the mutations is consistent with the increased frequency of his^{-} transformants obtained with double-strand breaks closer to the chromosomal mutations (Tables 1 and 2).

An additional unexpected class of pSZ515 *Kpn* I/*Xho* I transformants was obtained in which the gap was only partially repaired. In four of the unstable URA^{+} HIS^{+} transformants (Fig. 4 *Upper*) and one of the unstable URA^{+} his^{-} transformants (data not shown), the plasmids lacked the fragment that extends between the *Pst* I site in *HIS3* and the *Pst* I site in *URA3* (Fig. 2). However, the *Pst* I site inside the gap had been regenerated, because the other flanking *Pst* I fragment (extending between *HIS3* and *ARS1*) was present. Further analysis of these plasmids showed that, although all contained the *Kpn* I site in-

Table 2. Phenotypes of pSZ515 transformant colonies

Plasmid	Site	$URA^{+u}HIS^{+u}$	$URA^{+u}his^{-}$	$URA^{+s}HIS^{+s}$	$URA^{+s}his^{-}$	$URA^{+u}HIS^{+s}$	$URA^{+s}HIS^{+u}$	% his^{-}
Uncut pSZ515	—	87	0	0	1	0	0	1
Kpn I-cut pSZ515	*HIS3*	54	15	37	13	0	0	24
Xho I-cut pSZ515	*HIS3*	122	0	37	9	0	0	5
Kpn I/*Xho* I-cut pSZ515	*HIS3*	69	5	29	17	0	0	18

Selection was for URA^{+}. For general procedures and abbreviations, see Table 1.

Table 3. Phenotypes of pSZ519 transformant colonies

Plasmid	Site	HIS^{+u}	HIS^{+s}
pSZ519 Uncut		56	0
Sma I-cut pSZ519	*sup3*	27	5

Selection was for HIS^+. For general procedures and abbreviations, see Table 1.

side the *HIS3* gene, they had deletions that removed the *Xho* I site in the *HIS3* fragment, one of the *Bam*HI junction sites, and the *Sal* I site in the pBR322 DNA (Fig. 4 *Lower*; data not shown for the URA^+ his^- plasmid). The deletions ranged in size from 0.9 kb to 1.8 kb. In these transformants, the gap has been only partially repaired from the *Kpn* I end. One possible explanation for the incomplete repair is that the *Xho* I end of the gap had been degraded past the region homologous to the *HIS3* gene. Ligation of the degraded end to the end from the partially repaired gap would result in a series of deletions extending from points in the *HIS3* fragment into plasmid sequences.

Since the repair of most double-strand breaks in yeast appears to require recombination (12–14), we tested whether the homologous overhanging ends of a restriction site could be ligated independently of recombination. Transformation with *Bgl* II-gapped pSZ212 results in both stable and unstable LEU^+ transformants, but 80–90% of these are his^- (Table 1). The his^- transformants could result either from ligation of the *Bgl* II overhanging ends to generate a 60-bp deletion or from repair of the 60-bp gap accompanied by gene conversion of the chromosomal mutations to the plasmid, as described above. By examining only the his^- transformants, we eliminate the contribution of any contaminating circular pSZ212 because uncut pSZ212 is HIS^+. This is particularly important because linear

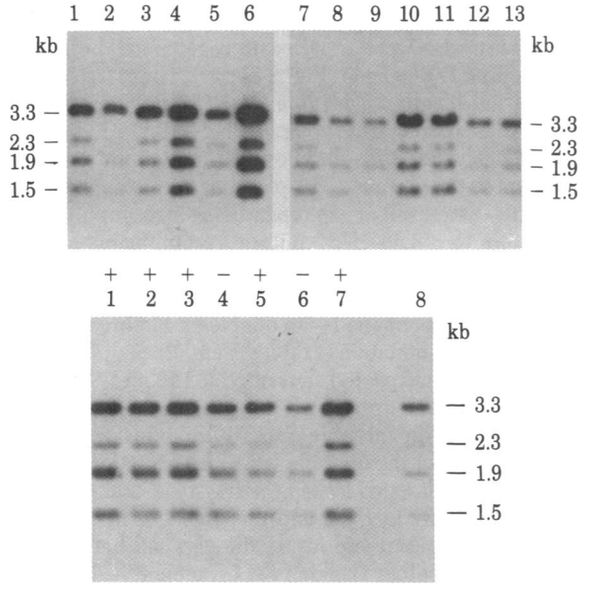

FIG. 3. Representative examples of repair of *Kpn* I/*Xho* I-gapped pSZ515. Yeast genomic DNA was purified from URA^+ transformants obtained by transforming D234-3B with *Kpn* I/*Xho* I-gapped pSZ515. Two micrograms of yeast DNA was digested with *Pst* I, electrophoresed on a 1% agarose gel, and transferred to nitrocellulose. The blots were hybridized to nick-translated pBR322. Fragment sizes were determined by comparison of mobilities with *Hin*dIII-digested λ DNA. All transformants shown give four *Pst* I fragments, thus the gap has been repaired. (*Upper*) Stable URA^+ transformants. Lanes: 1 and 13, pSZ515 standard; 2–6, HIS^+ transformants; 7–12, his^- transformants. (*Lower*) Unstable URA^+ transformants. Lanes: 8, pSZ515 standard; 1–7, HIS phenotype as labeled.

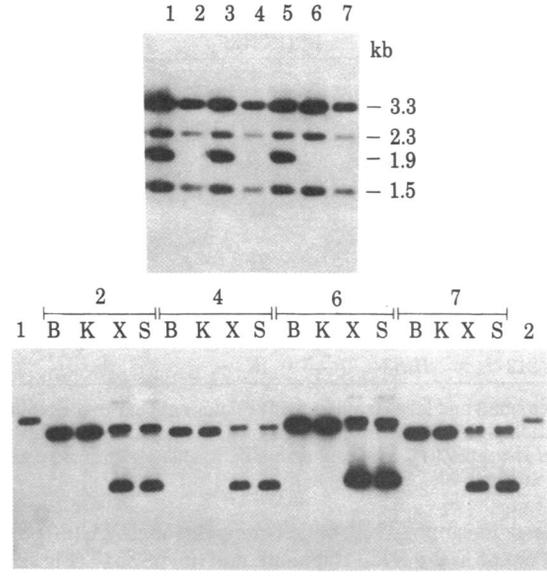

FIG. 4. Partial repair of *Kpn* I/*Xho* I-gapped pSZ515. (*Upper*) DNA isolated from unstable URA^+ HIS^+ transformants of D234-3B and *Kpn* I/*Xho* I-gapped pSZ515 was digested with *Pst* I and analyzed as in Fig. 2. Lanes: 1, pSZ515 standard; 2–7, transformant samples. (*Lower*) DNA from the transformants shown in lanes 2, 4, 6, and 7 (*Upper*) was cut with *Bam*HI, *Kpn* I, *Sal* I, or *Xho* I and analyzed as in Fig. 2, except that electrophoresis was on a 0.5% agarose gel. Lanes: 1 and 2, linear pSZ515 standard; B, *Bam*HI; K, *Kpn* I; S, *Sal* I; X, *Xho* I. In all four transformants, *Bam*HI cleaves the plasmid only once, generating a linear fragment of the same size as *Kpn* I digestion. This fragment is smaller than the pSZ515 standard. In the *Xho* I and *Sal* I digests, the plasmid DNA is not digested; bands corresponding to supercoiled and nicked plasmid DNA are present. The faint linear band may have resulted from shearing of plasmid yeast DNA during DNA isolation.

or gapped replicating plasmids transform at lower frequencies than do circular replicating plasmids (unpublished data). Twenty-six unstable LEU^+ his^- transformants were shown to contain a *Bgl* II site and must therefore result either from gap repair or religation (data not shown). his^- transformants resulting from religation will lack the 60-bp *Bgl* II fragment. We determined whether or not the his^- transformants contained the 60-bp *Bgl* II fragment by Southern blotting experiments using the isolated 5′-end labeled *Bgl* II fragment from *HIS3* as a hybridization probe. We found that 8 out of 26 his^- plasmids had homology to the probe while the others did not (Fig. 5). Therefore, his^- transformants in this case result from both religation of the *Bgl* II ends and gene conversion.

DISCUSSION

The repair of a double-strand gap represents a pathway for gene conversion. We have proposed that initiation of recombination by a double-strand break followed by gap repair may be a mechanism for meiotic gene conversion (3, 15). Meiotic gene conversion events are associated with reciprocal exchanges (4). After gap repair on plasmids, we recover both crossover and noncrossover products. Therefore in this respect gap repair on plasmids in mitotic cells is similar to gene conversion in meiosis. Accurate quantitation of the percentage of integrated versus nonintegrated plasmids is difficult because of the possibility of multiple events. However the two classes of plasmids are produced at approximately equal frequencies. We have previously shown that the repair of a double-strand break or gap requires the *RAD52* gene product (1); this is also required for meiotic and mitotic gene conversion (16, 17). Additional genetic ar-

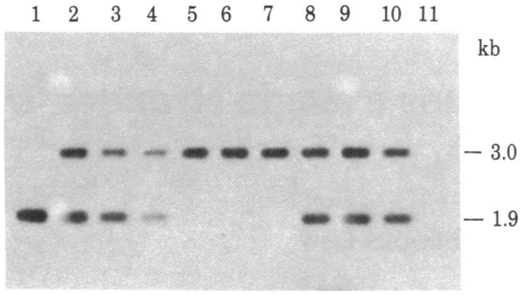

FIG. 5. Religation of *Bgl* II-gapped pSZ212. DNA was purified from unstable LEU$^+$ his$^-$ transformants obtained by transforming LL20 with *Bgl* II-cut pSZ212. The DNA was digested with *Pst* I; Southern blots were hybridized to the 60-bp *Bgl* II *HIS3* fragment. Lanes: 1, pSZ212 standard; 2–4, HIS$^+$ transformants; 5–10, his$^-$ transformants; 11, pSZ511 standard. The probe hybridizes to the correct pSZ212 *Pst* I fragment but does not hybridize to the deletion-derivative pSZ511. The 3.0-kb band present in transformant samples corresponds to the chromosomal *HIS3* fragment. While all the HIS$^+$ transformants contain the *Bgl* II *HIS3* fragment, three of the his$^-$ transformants lack homology to the probe. We analyzed 20 additional his$^-$ transformants; 15 of these did not contain the *Bgl* II *HIS3* fragment.

guments suggesting that meiotic recombination is initiated by double-strand breaks and that gene conversion is mediated by double-strand gap repair are discussed elsewhere (3).

Genetic events which occur in DNA flanking a region of gap repair are of interest because they may help to define the mechanism of the repair process. We have observed gene conversion in DNA flanking a double-strand gap, and our experiments suggest that these conversion events can result both from repair of heteroduplex DNA flanking the gap and from repair of a gap enlarged by degradation. In the unstable LEU$^+$, stable HIS$^+$ pSZ511 transformants the chromosomal mutations outside of the gap were converted to wild type, while a repaired, replicating plasmid was produced. These transformants are most simply explained as resulting from repair of heteroduplex DNA formed in the region adjacent to the repaired gap. However the low frequency of this class of transformants makes it unlikely that all of the transformants in which the chromosomal mutations were transferred to the plasmid arose by repair of heteroduplex DNA. Several experiments indicate that linear plasmid DNA can be degraded, such that gene conversion could occur by repair of the enlarged gap. Transformants obtained from replicating plasmids that have breaks or cuts in pBR322 DNA which lack homologous overhanging ends often contain deletions around the site of the break (unpublished results). The pSZ515 plasmids in which the gap is only partially repaired appear to have been degraded at one end past the region of homology to yeast DNA.

Most repair of chromosomal double-strand breaks formed by γ irradiation of yeast has been shown to require *RAD52* and the presence of homologous DNA (12–14). Our observation that complementary overhanging DNA ends on a plasmid transformed into yeast can be efficiently religated in a recombination independent reaction suggests that an additional mechanism for the repair of double-strand breaks may exist. Such repair could occur by ligation of homologous ends or degradation of nonhomologous ends until sufficient homology was exposed to permit ligation. Healing of the double-strand break generated at *MAT* during switching has been observed to occur in 1% of *rad52* cells. The healed chromosomes contain deletions around *MAT* (18). When we transformed with plasmids cut at a single site or when we did not select for repair of a gap containing homologous ends approximately 70% of the transformants had nonintegrated plasmids (Table 1). This increase in replicating plasmids over the 50% observed when we selected for correct gap repair probably results from ligation of the homologous ends. We have never observed ligation of homologous ends on a gapped integrating plasmid (1, 19). Since circular plasmids integrate at much lower frequencies than do linear plasmids, ligated integrating plasmids presumably rarely integrate and thus are undetected.

The observed behavior of gapped replicating plasmids suggests a rapid method for the recovery of chromosomal alleles. We have previously shown that if a gap on a plasmid includes the region homologous to a chromosomal mutation the mutation will be transferred to the plasmid by gap repair (2). Our experiments demonstrate that gene conversion of chromosomal information onto extrachromosomal plasmids occurs at a high frequency. The repaired autonomous plasmid containing the chromosomal allele can be subsequently directly transformed into *E. coli*. An additional application of these results is that replicating plasmids can be stimulated to integrate by making a double-strand break in yeast sequences on the plasmid prior to transformation.

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